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First cultivation of health-associated *Tannerella* sp. HOT-286 (BU063)

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Abstract

Despite significant advances in recent years in culture-independent molecular microbiology methods, the detailed study of individual bacterial species still relies on having pure cultures in the laboratory. Yet over a third of the approximately 700 different bacterial taxa found in the human oral cavity are as-yet-uncultivated *in vitro*. One such taxon, *Tannerella* sp. HOT-286 (phylotype BU063), is the focus of much interest since it is associated with periodontal health, while *Tannerella forsythia*, its closest phylogenetic neighbour, is strongly associated with periodontal disease. HOT-286, however, has remained uncultivated despite the efforts of several research groups, spanning over a decade. The aim of this study was to cultivate *Tannerella* sp. HOT-286. A heavily-diluted sample of subgingival plaque was inoculated onto culture plates, supplemented with siderophores (pyoverdines-Fe-complex or desferricoprogen) or a neat plaque suspension. After eight days of anaerobic incubation, microcolonies and colonies showing satellitism were passaged onto fresh culture plates cross-streaked with potential helper strains or onto cellulose-acetate membranes placed over lawn cultures of helper strains. Sub-cultured colonies were identified by 16S rRNA-gene-sequencing, and purity confirmed by sequencing 20 clones/library prepared from a single colony. Three of the colonies of interest (derived from pyoverdines- and plaque-supplemented plates) were identified as *Tannerella* sp. HOT-286. The isolates were found to be incapable of independent growth, requiring helpers such as *Propionibacterium acnes* or *Prevotella intermedia* for stimulation, with best growth on membranes over ‘helper’ lawns. A representative isolate was subjected to phenotypic characterization and found to produce a range of glycosidic and proteolytic enzymes. Further comparison of this novel ‘periodontal health-associated’ taxon with *T. forsythia* will be valuable in investigating virulence factors of the latter, and possible health benefits of the former.

Introduction

Although the advent of next-generation sequencing has revealed the true diversity of the human oral microbiome, the need for laboratory culture for the comprehensive physiological and pathological characterization of individual bacterial species remains. Approximately 700 bacterial taxa/species have been identified in the human oral cavity, based on 16S rRNA gene sequence data (Human Oral Microbiome Database, HOMD, release 13 (www.homd.org)). However of these, nearly 250 are as-yet-uncultivated *in vitro* (Chen et al. 2010; Dewhirst et al. 2010). Examples of uncultivated oral bacterial taxa include all members of the candidate bacterial Divisions SR1 and GN02 (Camanocha and Dewhirst 2014). Until recently, there were also no cultivated oral phylotypes from the phyla TM7 and *Chloroflexi* – a single oral strain from each phylum has now been successfully cultivated (He et al. 2015; Vartoukian et al. 2016). .

The recently cultivated TM7 strain, TM7x, has a reduced genome of 705 kb which lacks the genes necessary for essential amino acid biosynthesis; consequently, it is incapable of independent growth and leads an obligately symbiotic relationship with another bacterium, *Actinomyces odontolyticus* (He et al. 2015). Davis et al (2013) have also shown that gene loss in bacteria is associated with auxotrophy for purine, pyrimidine, fatty acid and amino acid synthetic pathways. Bacteria that are metabolically dependent on others may be impossible to grow in pure culture. Conversely, in-vitro cultivation of bacteria in consortia can enable the isolation of previously uncultivated bacteria (Tanaka and Benno 2015; Vartoukian et al. 2010). In particular, species within biofilm communities, such as dental plaque, may depend on each other for metabolic cooperation and intercellular signals (Mihai et al. 2015; Stewart 2012; Vartoukian et al. 2010). Kummerli and co-workers (Kummerli et al. 2009; Kummerli et al. 2014) have reported that the sharing of metabolites such as iron-scavenging siderophores is particularly prevalent in structured bacteria-host environments. It has been

suggested that ‘unculturable’ bacteria may have lost the ability to produce siderophores (Lewis et al. 2010), and depend on provision from neighboring bacteria. Indeed, there is evidence that adding siderophores to culture media stimulates the growth of previously uncultivated organisms (D'Onofrio et al. 2010; Guan and Kamino 2001; Vartoukian et al. 2016).

Tannerella forsythia is strongly associated with periodontitis (Socransky et al. 1998), possesses several virulence factors (Sharma 2010) and is the only cultivable taxon from the genus *Tannerella*. The as-yet-uncultivated oral phylotype *Tannerella* sp. HOT-286 (clone BU063) is phylogenetically closely-related to *T*. *forsythia,* but is associated with periodontal health rather than disease (de Lillo et al. 2004; Kumar et al. 2003; Leys et al. 2002). Fodor *et al* (2012) included *Tannerella* sp. HOT-286 on their high-priority microorganisms ‘most-wanted’ for genome sequencing list; de Lillo et al (2004) suggested over a decade ago that work to enable culture of *Tannerella* sp. HOT-286 should be urgently prioritised.

The aim of this study was to cultivate the previously-uncultivated oral phylotype *Tannerella* sp. HOT-286, using several approaches: growth in consortia, addition of siderophores, cross streaking with helper strains, and growth on membranes over helper lawns.

Materials and methods

Ethical approval for the study was granted by the South West London REC 3 Research Ethics Committee (REF: 10/H0803/161). A 50-year-old female subject with chronic periodontitis, who had not received periodontal or antimicrobial therapy within the previous three months, was recruited for the study with her informed consent. Subgingival plaque was

collected with a sterile curette from two deep (7-8 mm) periodontal pockets, pooled and suspended in Reduced Transport Medium (RTM) (Bowden and Hardie 1971).

The sample was transported within 45 min of collection to an anaerobic workstation (Don Whitley Scientific Ltd.) with an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide at 37°C. It was diluted to 10^{-6} in RTM after vortexing for 1 min, and 50 µl of the diluted plaque suspension was used to inoculate multiple pre-reduced Blood Agar Base No.2 (Lab M, UK) / 5% horse blood (BA) plates. A well was made in the center of each agar plate to which was added 150 µl of 0.1 mg/ml solutions of pyoverdines-Fe-complex (Sigma-Aldrich, UK) or desferricoprogen (EMC Microcollections, Germany), 150 µl of neat plaque suspension or 150 µl sterile water.

After eight days of anaerobic incubation, the mixed cultures were inspected under a plate-dissecting microscope for microcolonies and colonies satelliting around/on larger colonies. The colonies of interest were passaged both onto BA plates, cross-streaked with potential helper strains (*Fusobacterium nucleatum subspecies polymorphum* NCTC 10562, or *Propionibacterium acnes* ATCC 6919), and onto 0.45 µm-pore cellulose acetate membranes (Sartorius, 1110650ACN) overlying fresh *F. nucleatum* or 48-hour *P. acnes* lawn cultures.

Growth on secondary plates was purified where necessary and DNA extracted using the GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, UK) with the protocol for Gram-positive bacteria, prior to endpoint PCR with ‘universal’ primers 27FYM and 1492R (Lane 1991) as described previously (Vartoukian et al. 2009). For secondary plates showing minimal growth, direct ‘touch’-PCR of single colonies with ‘universal’ primers (Vartoukian et al. 2009) was performed. PCR products were subjected to partial 16S rRNA gene sequencing using primer 519R (Vartoukian et al. 2009).

For cultures identified as *Tannerella* sp. HOT-286 (phylotype BU063), purity was confirmed by sequencing 20 cloned inserts from a library prepared (as described previously (Vartoukian et al. 2009)) using the amplification product of 16S rRNA gene 'touch'-PCR of a single colony with 'universal' primers. Subsequently, the full length of the 16S rRNA gene was sequenced with multiple primers for triple coverage (Vartoukian et al. 2009).

Colonial and cellular morphology of *Tannerella* sp. HOT-286 strains were determined by: examination under a dissecting microscope, light microscopy after Gram staining and transmission electron microscopy (TEM). For TEM, isolated colonies were gently suspended in 10 mM Tris-HCL buffer (pH 7.4) at a concentration of about 10^8 cells per ml. Samples were negatively stained with 1% (wt/vol) phosphotungstic acid (pH 6.5) for 20 to 30 s. The specimens were examined with a JEOL model JEM-1200EX transmission electron microscope (JEOL USA, Inc., Peabody, MA) operating at 100 kV.

Enzyme profiles were determined for *Tannerella* sp. HOT-286 isolate SP18_24 and *T. forsythia* FDC 338^T using the API ZYM test (BioMerieux, France) and the Rapid ID 32 A anaerobe identification kit (BioMerieux, France) in duplicate.

Susceptibility to penicillin (1 unit), amoxicillin (10 µg), ampicillin (2 µg), erythromycin (5 µg), tetracycline (10 µg), metronidazole (5 µg), ceftazidine (30 µg), gentamycin (10 µg), chloramphenicol (10 µg) and ciprofloxacin (1 µg) (Oxoid, UK) was determined in duplicate using the disc diffusion method for: a) *Tannerella* sp. HOT-286 SP18_24, using cultures cross-streaked with *P. acnes*, and b) *T. forsythia* FDC 338^T.

Growth characteristics of *Tannerella* sp. HOT-286 SP18_24 were investigated as follows, with duplicate testing in all cases:

The ability of pyoverdines-Fe to stimulate growth of this strain on BA under anaerobic conditions was assessed: a) by adding either 150 µl of 0.1mg/ml pyoverdines-Fe or an equivalent volume of sterile water (negative control) to a central well on the plates, or b) by applying a small circular inoculum of live *P. acnes* (positive control) to the center of the plates.

The effect on growth of SP18_24 of *P. acnes* culture supernatant (CS) or cell-free extract (CFE) was assessed using a method similar to that described above, with addition of test (CS or CFE) or negative control (Nutrient Broth no. 2 (NB; Oxoid, UK) or PBS) agents to a central well, or inoculation with live *P. acnes* as positive control. CS was prepared from a 4-d NB culture of *P. acnes* by centrifuging the culture and passing the supernatant through a 0.2 µm-pore filter. CFE was prepared from the same 25 ml broth culture by re-suspending the pellet in 5 ml PBS, sonicating the suspension for three pulses of 2 min, centrifuging and filtering the supernatant.

The effect of CS/CFE on growth of SP18_24 was also assessed in broth culture. Briefly, SP18_24 was cultured in NB + 1% yeast extract, with or without: CS (50%, v/v), CFE (25%, v/v) or equivalent volumes of plain NB or PBS as controls. Growth was assessed over 16 d using spectrophotometric turbidity measurements at 600 nm.

Finally, a panel of seven oral bacteria was evaluated alongside *P. acnes* for their stimulatory effect on the growth of SP18_24 as lawn cultures on BA. Small circular inocula of the following bacterial strains were applied to plates: *Streptococcus oralis* (NCTC 7864), *Veillonella dispar* (NCTC 11831), *Actinomyces oris* (ATCC 19246), *Parvimonas micra* (ACTC 33270), *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), *P. acnes* (ATCC 6919) and *F. nucleatum (*NCTC 10562). After 7 d of anaerobic incubation, growth stimulatory effect was graded arbitrarily as 0, +, ++ or +++.

Results

A heavily-diluted subgingival plaque sample was inoculated onto culture plates supplemented either with siderophores or a neat suspension of the plaque sample. Forty-six isolates forming microcolonies or exhibiting satellite growth around other colonies were passaged to fresh plates cross-streaked with helper strains and onto membranes overlying lawn cultures of helpers. Three isolates (two from a pyoverdines-Fe supplemented plate and one from a plaque-supplemented plate) formed several large, cream-coloured colonies on membranes overlying *P. acnes* lawns but showed no, or limited, growth (1-2 tiny colonies) on: (i) secondary plates cross-streaked with *F. nucleatum* and *P. acnes* as helpers, or (ii) membrane cultures over *F. nucleatum* lawns. The isolates were identified as *Tannerella* sp. HOT-286 (phylotype BU063); and the cultures were confirmed pure by sequence analysis of multiple cloned amplicons derived from a single colony of each isolate. The full-length 16S rRNA gene sequences of the three isolates were found to be identical, and 99.2% similar over 1450 bases to *Tannerella* clone BU063 accession number AY008308. The novel sequences were deposited in the GenBank nucleotide sequence database with the following accession numbers: *Tannerella* sp. HOT-286 isolate SP18_4 - KT861600; *Tannerella* sp. HOT-286 SP18_24 - KT861601; and *Tannerella* sp. HOT-286 SP18_26 - KT861602.

The three isolates showed limited independent growth (Figure 1 A), and were significantly stimulated by *P. acnes*, but not *F. nucleatum* (Figure 1 B). Culture on membranes over *P. acnes* lawns resulted consistently in stronger growth than was observed after culture directly on media with *P. acnes* cross-streaks (Figure 1 B and C). The isolates were successfully revived after storage in broth/glycerol at -80°C, although growth was initially sparse, consisting of tiny colonies approximately 0.2 mm in diameter and larger, 1-1.5 mm colonies

of the same type (Figure 1 D). After two passages, colonies of *Tannerella* sp. HOT-286 on BA measured, on average, approximately 0.5 mm in diameter after 8 d growth, had a circular or slightly irregular shape, undulate edge, convex profile and convoluted surface. Colonies were grey/off-white in colour, and speckled with opaque cream internal flecks.

Gram-staining and transmission electron microscopy (TEM) revealed that cells of *Tannerella* sp. HOT-286 were Gram-negative and filamentous (Figure 1 E), measuring 1.2 µm in width, and comprised of segments of varying sizes (Figures 1 F and G). Cells ranged in length from 4 to over 50 µm (Figure 1 F). Pili, flagella, or other surface structures were not observed (Figures 1 F and G).

There was insufficient independent growth of *Tannerella* sp. HOT-286 SP18_24 on BA to perform API ZYM and Rapid ID 32 A tests according to the manufacturer's instructions. Therefore, tests were repeated using SP18_24 biomass harvested from 7-d cultures cross-streaked with *P. acnes*, and compared against results of equivalent tests for *P. acnes*. For some tests, the result was positive for SP18_24 but negative for *P. acnes*, and vice versa, (Table 1), lending credence to the validity of the results. SP18_24 exhibited proteolytic and glycolytic activity and was positive for alkaline phosphatase, acid phosphatase, esterase, esterase lipase and Naphthol-AS-BI-phosphohydrolase.

Tannerella sp. HOT-286 SP18_24 was susceptible to amoxicillin, ampicillin, erythromycin, tetracycline, metronidazole and ceftazidine (with zones of inhibition measuring 30 mm or more in diameter); weakly susceptible to penicillin and chloramphenicol (zones of inhibition of 14-20 mm); and resistant to gentamycin and ciprofloxacin (no zone). The antimicrobial susceptibility profile for *T. forsythia* FDC 338^{T} was the same as that of SP18_24 except that it was strongly susceptible to penicillin, with a 65 mm zone of inhibition.

Neither pyoverdines-Fe, nor the CS or CFE of *P. acnes* showed any stimulatory effect on the growth of SP18_24 relative to negative controls. Furthermore, SP18_24 did not grow in broth culture, with or without *P. acnes* CS/CFE.

P. acnes and *P. intermedia* showed the strongest growth stimulation (+++) of SP18_24, with dense satelliting growth around *P. acnes* (Figure 4 g), and the development of large colonies of SP18_24 at a distance of up to 25 mm from *P. intermedia* as well as satellite growth (Figure 4 f). *A. oris* and *F. nucleatum* showed moderate growth stimulation (++, Figures 4 d & h). *V. dispar* and *P. gingivalis* were able to weakly stimulate growth of SP18_24 (+, Figures 4 b & e), whereas *S. oralis* and *P. micra* showed no stimulatory capacity (Figures 4 a & c).

The novel *Tannerella* sp. HOT-286 strains have been deposited in culture collections as follows: *Tannerella* sp. HOT-286 SP18_4 – DSMZ XX, JCM 31301; *Tannerella* sp. HOT-286 SP18_24 – DSMZ XX, JCM 31302; and *Tannerella* sp. HOT-286 SP18_26 – DSMZ XX, JCM 31303.

Discussion

Several authors have highlighted the need to cultivate *Tannerella* sp. HOT-286 (de Lillo et al. 2004; Leys et al. 2002; Zuger et al. 2007). Although it was reported that *Tannerella* sp. HOT-286 had been successfully cultivated as part of a consortium (Duran-Pinedo et al. 2011), the consortium was lost before a pure culture could be obtained (Frias-Lopez, personal communication). In this study, we used growth in consortia, growth with a cross-streaked helper organism, growth on a membrane over a helper organism lawn, and

supplementation with siderophores to allow culture of *Tannerella* sp. HOT-286, leading to its successful isolation in purity. Two of the three novel *Tannerella* isolates were cultured on pyoverdine-Fe-supplemented plates, although growth stimulation by pyoverdine-Fe was not confirmed. Pyoverdine-Fe has been shown to be strongly stimulatory to the difficult-to-culture bacterium *Prevotella* sp. HOT-376 (Vartoukian et al. 2016), demonstrating that growth enhancement by siderophores is a selective phenomenon.

Tannerella sp. HOT-286 was found to be dependent for growth on the proximity of a helper strain, *P. acnes*. It has been observed over the years within the Wade and other labs (Davis et al. 2014) that *P. acnes* stimulates the growth of a number of previously-uncultivated bacteria, although the mechanisms of action are unknown. Interestingly, although co-culture with live *P. acnes* had a strong growth-promoting effect on *Tannerella* sp. HOT-286, this effect was not observed with *P. acnes* CS or CFE, suggesting that the stimulating factor is labile. Furthermore, growth was enhanced more strongly by culturing *Tannerella* sp. HOT-286 on the surface of a membrane over a lawn culture of *P. acnes*, than by culturing the strain directly on agar with *P. acnes* cross-streaks. This would imply either that a greater amount of 'helper' signal, as provided by the larger surface area of a lawn culture than of a narrow cross-streak, is needed for growth; or that separation from the agar surface by a membrane helps protect the recipient from potential growth inhibitors present in the agar medium. It has been shown that hydrogen peroxide, produced during autoclave sterilisation of media which includes both phosphate and agar can inhibit bacterial growth (Tanaka et al. 2014).

Six of eight oral bacterial species, representing four phyla, stimulated the growth of *Tannerella* sp. HOT-286 SP18_24. In general, growth stimulation was observed as satellitism immediately surrounding the helper strain, although *P. intermedia* effected the

295 emergence of several large outlier colonies of SP18_24 at a distance from the helper.
296 Although beyond the scope of this study, a future challenge will be to determine by what
297 mechanism these different helpers stimulate growth of SP18_24, and whether there is a
298 universal or specific mode of action.

299

300 Cells of *Tannerella* sp. HOT-286 were found to be segmented filaments of variable length,
301 confirming the observations of Zuger *et al* (2007) following FISH analysis of BU063 cells.
302 However, their impression that individual segments of cells are of equal length and that
303 consequently, overall cell length is a reflection of the total number of segments present, was
304 not confirmed by our TEM images: whereas the 16 µm cell shown in Figure 1 G had six
305 segments, the longer 50 µm cell in Figure 1 F had only four.

306

307 *Tannerella* sp. HOT-286 is found in high prevalence, but low abundance, in periodontal
308 disease-associated plaques (Zuger et al. 2007), and the relative abundance of this phylotype
309 is estimated to be around 0.05% of subgingival bacteria (HOMD release 13) (Beall et al.
310 2014). Evidence from several studies has indicated that, unlike *T. forsythia*, *Tannerella* sp.
311 HOT-286 is primarily associated with periodontal health (de Lillo et al. 2004; Kumar et al.
312 2003; Leys et al. 2002); Leys et al (2002) reported odds ratios for prevalence in periodontitis
313 of *Tannerella* sp. HOT-286 and *T. forsythia* of 0.1 and 9.9 respectively. The apparent
314 phenotypic dichotomy between these closely-related taxa is clearly of interest, and
315 comparative studies of *Tannerella* sp. HOT-286 and *T. forsythia* could provide some insight
316 into factors involved in the latter's virulence.

317

318 To this end, an enzymatic profile of *Tannerella* sp. HOT-286 was generated and compared
319 to that of *T. forsythia*. The profiles of the two *Tannerella* taxa were similar, despite their
320 different clinical phenotypes. Both taxa produced a range of proteolytic, hydrolytic, lipolytic

and saccharolytic enzymes. A comparison of the enzyme activity of the two taxa did not reveal any obvious differences relevant to the virulence of *T. forsythia* although clearly its virulence could be related to factors unconnected to the tests included in the API ZYM and Rapid ID 32 A kits. Antimicrobial susceptibility profiles were also similar for the two taxa.

Beall and co-workers (2014) isolated individual cells of *Tannerella* sp. HOT-286 by flow cytometry and used multiple displacement amplification to generate a collection of single-cell-amplified genomes with predicted sizes from 3.44 to 4.07 Mb. Putative virulence genes of *T. forsythia* were detected by comparative analysis with the HOT-286 genomes and included genes encoding the PrtH, BspA, NanH and KLIKK proteases (Beall et al. 2014; Ksiazek et al. 2015). Beall *et al* (2014) reported a surprisingly high level of strain polymorphism and substantial nucleotide divergence between the various genomes of *Tannerella* sp. HOT-286. Given that multiple displacement amplification can result in uneven amplification of the genome (Lasken 2012), complete genome sequences are being generated for the three *Tannerella* sp. HOT-286 strains isolated in this study, to enable further comparative genomic analysis with *T. forsythia*.

Leys and co-workers (2002) showed that subgingival plaque samples were less likely to be dual-colonized with *Tannerella* sp. HOT-286 and *T. forsythia* than would be expected by chance. They suggested a specific exclusionary mechanism, with the possibility that *Tannerella* sp. HOT-286 may provide protection from acquisition of *T. forsythia*. Inverse associations between oral bacteria as a result of antagonistic interactions have been reported for *Streptococcus mutans* and *Streptoccous sanguinis* (Kreth et al. 2005). If confirmed, this could have far-reaching implications in the management of periodontitis. With health-associated *Tannerella* sp. HOT-286 having finally been cultivated, and available for study, such exciting therapeutic possibilities may now be explored.

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Figure legends

Figure 1: Growth characteristics, colony and cellular morphology of *Tannerella* sp. HOT-286. (A-C) Six-day cultures of *Tannerella* sp. HOT-286: (A) No visible growth in absence of helper strain; (B) Satellitism beside *P. acnes* streak on left, no growth beside *F. nucleatum* streak on right; (C) Strong growth on membrane over *P. acnes* lawn. (D) Nine-day culture of *Tannerella* sp. HOT-286 after revival from -80 °C storage showing sparse growth of colonies of variable size; Arrows indicate position of tiny colonies; Bar = 5 mm. (E) Cellular morphology of *Tannerella* sp. HOT-286 by Gram staining; Bar = 10 µm. (F-G) Transmission electron photomicrographs of 2 cells of *Tannerella* sp. HOT-286 (Strain SP18_24): (F) Long cell of about 50 µm in length; Bar = 5 µm. (G) Cell showing segments of variable size; Bar = 2 µm.

Figure 2: Seven-day cultures of *Tannerella* sp. HOT-286 (SP18_24) showing growth stimulation by several of the eight potential helper strains tested: (a) *Streptococcus oralis*, (b) *Veillonella dispar*, (c) *Parvimonas micra*, (d) *Actinomyces oris*, (e) *Porphyromonas gingivalis*, (f) *Prevotella intermedia*, (g) *P. acnes* and (h) *F. nucleatum*.

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	Tannerella sp. HOT-286 (SP18_24) [2]	*Tannerella forsythia* [T] (FDC 338)	*Propionibacterium acnes* (ATCC 6969)
API ZYM			
Alkaline phosphatase	+	+	-
Esterase (C4)	+	+	-
Esterase lipase (C8)	+	+	-
Lipase (C14)	-	-	-
Leucine arylamidase	+	+	-
Valine arylamidase	-	-	-
Cystine arylamidase	-	-	-
Trypsin	+	+	-
α-chymotrypsin	-	-	-
Acid phosphatase	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	-
α-galactosidase	-	-	-
β-galactosidase	-	-	+
β-glucuronidase	-	+	-
α-glucosidase	+	-	-
β-glucosidase	-	-	-
N-acetyl-β-glucosaminidase	-	+	+
α-mannosidase	-	-	+
α-fucosidase	-	+	-
Rapid ID 32 A			
Urease	-	-	-
Arginine dihydrolase	-	-	+
α-galactosidase	-	-	-
β-galactosidase	-	+	+
β-galactosidase-6-phosphate	-	+	-
α-glucosidase	+	-	-
β-glucosidase	-	+	-

α-arabinosidase	-	-	-
β-glucuronidase	-	-	-
N-acetyl-β-glucosaminidase	-	+	+
Mannose fermentation	-	-	+
Raffinose fermentation	-	-	-
Glutamic acid decarboxylase	-	-	-
α-fucosidase	+	+	-
Reduction of nitrates	-	-	+
Indole	+	-	+
Alkaline phosphatase	+	+	-
Arginine arylamidase	+	+	+
Proline arylamidase	-	-	+
Leucyl glycine arylamidase	+	+	-
Phenylalanine arylamidase	+	-	-
Leucine arylamidase	+	+	-
Pyroglutamic acid arylamidase	+	-	-
Tyrosine arylamidase	+	+	-
Alanine arylamidase	+	+	+
Glycine arylamidase	-	-	+
Histidine arylamidase	+	+	-
Glutamyl glutamic acid arylamidase	-	-	-
Serine arylamidase	-	-	+

Table 1. Results of API ZYM and rapid ID 32 A tests [1]

[1] Reactions graded on a scale of 0-5 with values of 3-5 reported as a positive result (+), as recommended in manufacturer's guidelines

[2] Cross-streaks of *P. acnes* present on source plates